
Recent clinical data have shown that early plasmin proteolysis of human fibrinogen results in the release of fibrinopeptide B (FPB) immunoreactivity into plasma. Upon thrombin treatment this activity increases indicating that the immunoreactivity arises from larger FPB-containing peptides. The presence of degradation products (from the B8 chain may be an index of fibrinogen proteolysis not only in normal hemostasis but also in pathological disorders such as thrombosis and disseminated intravascular coagulation. Therefore the capability of detecting events leading to progressive plasmin degradation at the B8 chain could be of major diagnostic and therapeutic significance. Toward this goal, we have isolated the various peptides released from human fibrinogen by plasmin proteolysis using HPLC on a reverse phase C18 column. These peptides were identified using amino acid analysis and radioimmunoassays for FPB. FPB 1-42 was the earliest fragment released during limited plasmin proteolysis. These levels reached a maximum and then began to fall during the course of the digestion. In addition, increasing levels of B8 1-21 and of FPB were shown to lag behind the production of B8 1-42. Using purified B8 1-21 and FPB the cleavage of the 15-42 bond. Exhaustive digestion yielded two major components, BP 1-14 and BP 22-42 and a minor component composed of BP 15-42. The rate of this reaction is not affected by the addition of hirudin indicating that it is not caused by trace amounts of thrombin. Taken together these data indicate a sequence of events in which B8 1-42 is initially cleaved from fibrinogen during plasm digestion. Free from its parent molecule, B8 1-42 can undergo further plasmin attack, preferentially at the 21-22 bond to yield B8 1-21 and B8 22-42. Plasmin then attacks B8 1-21 at the 14-15 bond to release FPB.


Thrombin cleaves fibrinogen to release fibrinopeptide A (FPA) and B (FPB) to form fibrin I and II respectively. Initially FPA is cleaved more rapidly than FPB but later the FPB cleavage increases and eventually passes that of FPA. Studies were made at 1.65 μM fibrinogen concentration 0.02U thrombin/ml, pH 7.4, 0.1% HCl. 37°. The initial FPA cleavage rate was 7 pmol/min and then increased to 70 pmol/min. The accelerative rate of cleavage was associated with fibrin polymerization as indicated by light scattering and absorbance measurements. Fibrin polymerization was inhibited by the synthetic tetrapeptide Gly-Pro-Arg-Pro. At a concentration of tetrapeptide of 23 μM (1400 fold molar ratio to fibrinogen) no detectable polymerization occurred over 180 min. At this tetrapeptide concentration the FPA cleavage rate was unaltered from that occurring without tetrapeptide but the rate of FPB cleavage did not show the acceleration normally associated with polymerization although virtually all the FPA was cleaved. The cleavage rates of FPA from fibrin I polymer and monomer were then compared. Fibrin I polymer was prepared by treating fibrinogen with trypsin and fibrin I monomer by similar treatment in the presence of excess tetrapeptide. The cleavage rate from monomer was similar to the initial slow cleavage rate when thrombin was added to fibrinogen. The cleavage rate of human fibrinogen was 25 fold more rapid than that from monomer. These data indicate that thrombin cleaves FPA slowly but completely from fibrinogen and at least 25-fold more rapidly from polymer. The data do not establish that thrombin cleaves FPB from fibrinogen.

FIBRINOPEPTIDE A CLEAVAGE AND PLATELET RELEASE IN WHOLE BLOOD IN VITRO. K.L. Kaplan. Department of Medicine, Columbia University College of Physicians & Surgeons, New York, NY, U.S.A.

The relationship between platelet release and fibrinopeptide A (FPA) cleavage from fibrinogen to form fibrin I was examined in blood allowed to clot in vitro. FPA and the platelet release products (glycoprotein IIb/IIIa) were quantitated by radioimmunoassay. In undisturbed blood platelet release and FPA cleavage occurred simultaneously. Addition of hirudin inhibited platelet release as well as FPA cleavage, indicating that thrombin was essential for platelet release. Addition of collagen increased platelet release and then FPA cleavage, and addition of ADP had a similar but less marked effect, suggesting that increased platelet activation accelerated thrombin formation. PGE1 and theophylline inhibited platelet release but did not affect FPA cleavage in the initial stages of incubation. When blood was aspirated, the effects of collagen and PGE1 and theophylline were qualitatively similar to those in undisturbed blood, but addition of hirudin no longer affected platelet release, suggesting that in this case platelet release occurred independently of thrombin action. Addition of dilute thrombin or tissue thromboplastin to undisturbed blood resulted in little effect on platelet release, indicating that exogenous thrombin or thrombin generated by the extrinsic system behaves differently from that generated by the intrinsic system. Finally, the thrombin concentrations were compared in platelet rich and platelet free plasma and found to be increased in platelet rich plasma in spontaneously clotting blood but not in blood, which was anticoagulated with thrombin added. These relationships suggest that thrombin formation via the intrinsic system occurs on the platelet surface leading to simultaneous platelet release and FPA cleavage, while thrombin generated via tissue thromboplastin leads to thrombin formation in the plasma and FPA cleavage precedes platelet release. Collagen primarily accelerates platelet release and secondarily increases thrombin formation.